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Tree Physiology review

Reactive oxygen species in *Hevea brasiliensis* latex and relevance to Tapping Panel Dryness

Yi Zhang, Julie Leclercq and Pascal Montoro[1]

CIRAD, UMR AGAP, F-34398 Montpellier, France; [1]Corresponding author (pascal.montoro@cirad.fr)

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Environmental stress can lead to oxidative stress resulting from an increase in reactive oxygen species (ROS) and involves redox adjustments. Natural rubber is synthesized in laticifers, which is a non-photosynthetic tissue particularly prone to oxidative stress. This paper reviews the current state of knowledge on the ROS production and ROS-scavenging systems in laticifers. These regulations have been the subject of intense research into a physiological syndrome, called Tapping Panel Dryness (TPD), affecting latex production in *Hevea brasiliensis*. In order to prevent TPD occurrence, monitoring thiol content appeared to be a crucial factor of latex diagnosis. Thiols, ascorbate and γ-tocotrienol are the major antioxidants in latex. They are involved in membrane protection from ROS and likely have an effect on the quality of raw rubber. Some transcription factors might play a role in the redox regulatory network in *Hevea*, in particular ethylene response factors, which have been the most intensively studied given the role of ethylene on rubber production. Current challenges for rubber research and development with regard to redox systems will involve improving antioxidant capacity using natural genetic variability.

Keywords: antioxidant, laticifer, redox, ROS scavenging, rubber tree.

Introduction

Latex cells amount to a unique cell factory involving redox systems. Among about 2500 latex-producing plant species, *Hevea brasiliensis* is the main source of natural rubber (NR), which accounts for 42% of total world consumption of rubber. The polymer *cis*-1,4-polyisoprene, known as NR, is synthesized in the rubber particles of laticifers, which are articulated and anastomosed latex cells (d'Auzac and Jacob 1989, de Faÿ and Jacob 1989*a*). Latex is the cytoplasm of these specialized tube cells. Laticifers are differentiated from vascular cambium (Figure 1A). The articulated laticiferous vessels are arranged in concentric rings in the phloem (Figure 1B). Latex flows out from the laticifers without mitochondria after cutting of the soft bark (tapping) (Figure 1C). For certain rubber clones with a low latex metabolism, application of an ethylene releaser (ethephon) to the bark stimulates latex flow and latex regeneration between two tappings (d'Auzac et al. 1997). Environmental and harvesting stresses, as well as the metabolic activity necessary for latex regeneration between two tappings, lead to the production of reactive oxygen species (ROS). Over-accumulation of ROS can lead to laticifer dysfunctions such as Tapping Panel Dryness (TPD). Tapping Panel Dryness halts latex flow (Figure 1D). The production and processing of NR have led to many studies on redox reactions and ROS-scavenging systems in laticifers, and on the supply of antioxidants to protect the rubber polymer.

Oxidation-reduction (redox) reactions involve a transfer of electrons between two compounds. Redox reactions are common and vital to some of the basic biological functions such as stress response, development, photosynthesis and respiration (Mittler 2002, You and Chan 2015). Redox homoeostasis is necessary to maintain a cell or compartment environment in favour of biological processes. A low level of ROS generation in the basal redox state of cells or tissues, e.g. $^1O_2$ (singlet oxygen), $O_2^{\bullet -}$ (superoxide radical), $^{\bullet}OH$ (hydroxyl radical) and H_2O_2 (hydrogen peroxide), is under the control of a ROS-scavenging system. Abiotic and biotic stress, as well as some plant

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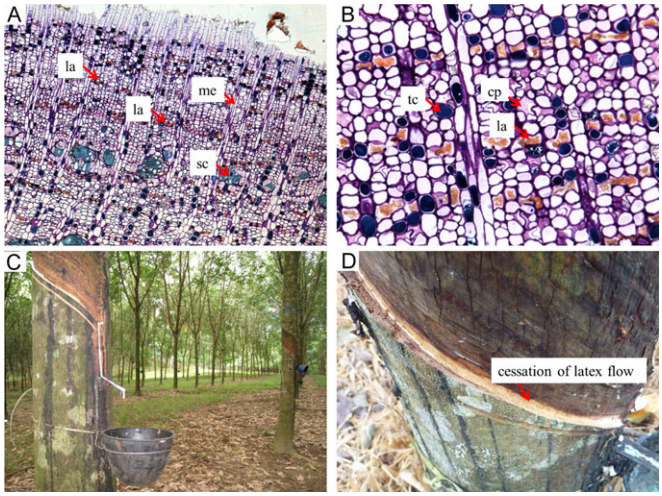

Figure 1. Illustration of laticifer anatomy, latex production and TPD symptoms. (A) Histological transversal section of phloem tissue (staining with oil-red O and toluidine blue, magnification ×5): (la) latex cells are stained in orange–red, (ca) cambium, (me) medullar ray, (cp) conducting phloem, (tc) tannin cell, (sc) sclereid (stone cell). (B) Histological transversal section of laticifer (staining with oil-red O, magnification ×20). (C) Normal latex flow after tapping. (D) Partial cessation of latex flow related to TPD.

development processes, are known to trigger disturbances in the basal redox state, which subsequently generates high levels of ROS. Peroxides and free radicals damage all components of the cell, including proteins, lipids and nucleic acids. The ROS are also involved in plant development and are also described as secondary messengers (Foyer and Noctor 2005, Baxter et al. 2014). The ROS-scavenging systems play an essential role in maintaining redox homoeostasis. Activities of antioxidant enzymes (superoxide dismutase (SOD), peroxidase, catalase (CAT) and glutathione reductase (GR)) and concentrations of antioxidant molecules (glutathione and ascorbate) are the most predominant functions in plants.

This paper sets out to review for the first time the documentation of ROS in latex cells with regard to rubber production and ROS-associated TPD. Finally, this paper surveys the inputs of research in terms of regulation of redox-related gene expression, genetic modification, genetic improvement and latex diagnosis for monitoring plantations.

ROS production and scavenging systems in laticifers

The types of ROS and their subcellular localization as well as ROS-scavenging enzymes in latex cells have been documented for a long time, and are summarized in Figure 2. The first reported source of ROS in latex was peroxidase (de Haan-Homans 1950). Then polyphenol oxidase (PPO) (Tata and Edwin 1970) and a specific PPO, *o*-diphenol oxidase (ODP) (Coupé et al. 1972), were reported. The main sources of ROS are produced by specific organelles (Table 1). Indeed, latex cells are non-photosynthetic cells harbouring specific compartments such as rubber particles, lutoids and Frey-Wyssling particles (de Faÿ et al. 1989). Frey-Wyssling particles are very specialized chromoplasts. These globules of 0.5–2 μm in diameter have a double membrane and contain lipids and carotenoids. These plastids have ODP, which are a source of ROS (Coupé et al. 1972). Lutoids are lysosomal micro-vacuoles of 1–3 μm in diameter, enclosed by a single membrane. They generally amount to 10–20% of the volume of fresh latex, and have been considered as the major source of ROS in latex cells (d'Auzac et al. 1989). The NADH-cytochrome *c* oxidoreductase activity was first measured in the membrane of isolated lutoids, but surprisingly that extract was not able to oxidize NADPH (Moreau et al. 1975). Lutoid membranous NADH-cytochrome *c*-reductase was likely to function as NADH-O_2 reductase, a generator of superoxide ions (d'Auzac et al. 1982). Enzymatic activity generating superoxide anions from NAD(P)H and O_2 was later observed (Cretin and Bangratz 1983). Lutoidic NAD(P)H oxidase generates species of toxic oxygen, which lead to peroxidatic degradation of the unsaturated lipids of the membrane (Chrestin et al. 1984). The NAD(P)H oxidase was reported as the main ROS source in laticifers, especially when the laticifers were under stress (Cretin and Bangratz 1983, Chrestin et al. 1984).

Redox homoeostasis is controlled by the biosynthesis and reduction of antioxidants and by ROS-scavenging enzymes. Latex contains three major antioxidants, namely thiol, ascorbate and tocotrienol. Some other molecules with antioxidant powers can be also detected, such as phytosterols, phospholipids, phenols, betaines, proteins and amino acids. The total thiol concentration is above 0.5–0.9 mM in latex (Jacob et al. 1984), and can reach up to 2.2 mM (Chrestin 1984). Up to 90% of them are glutathione and cysteine (McMullen 1960). Cysteine is an important biochemical precursor for glutathione synthesis (Franklin et al. 2009). Glutathione and cysteine are the main thiols in latex (McMullen 1960). Total thiols provide a powerful reductive pool in latex (McMullen 1960). The total thiol content is one parameter of latex diagnosis, which is positively correlated with latex production and is used to monitor the physiological status of trees under production (Eschbach et al. 1984, Prevot et al. 1984*b*, Sreelatha et al. 2009).

The concentration of ascorbate can range from 1.9 to 3.9 mM in latex (Archer et al. 1969, Chrestin 1984). The ascorbate and glutathione biosynthesis pathways have been partially characterized (Yujie 2011, Putranto et al. 2012). D-mannose/L-galactose pathway is the most significant source of ascorbate in plants. GDP-L-galactose phosphorylases and GDP-D-mannose-3′, 5′-epimerase are important enzymes related to this pathway (Ishikawa and Shigeoka 2008). Two genes encoding GDP-L-galactose phosphorylases were upregulated during the first five tappings of re-opened rubber trees in this pathway (Yujie 2011).

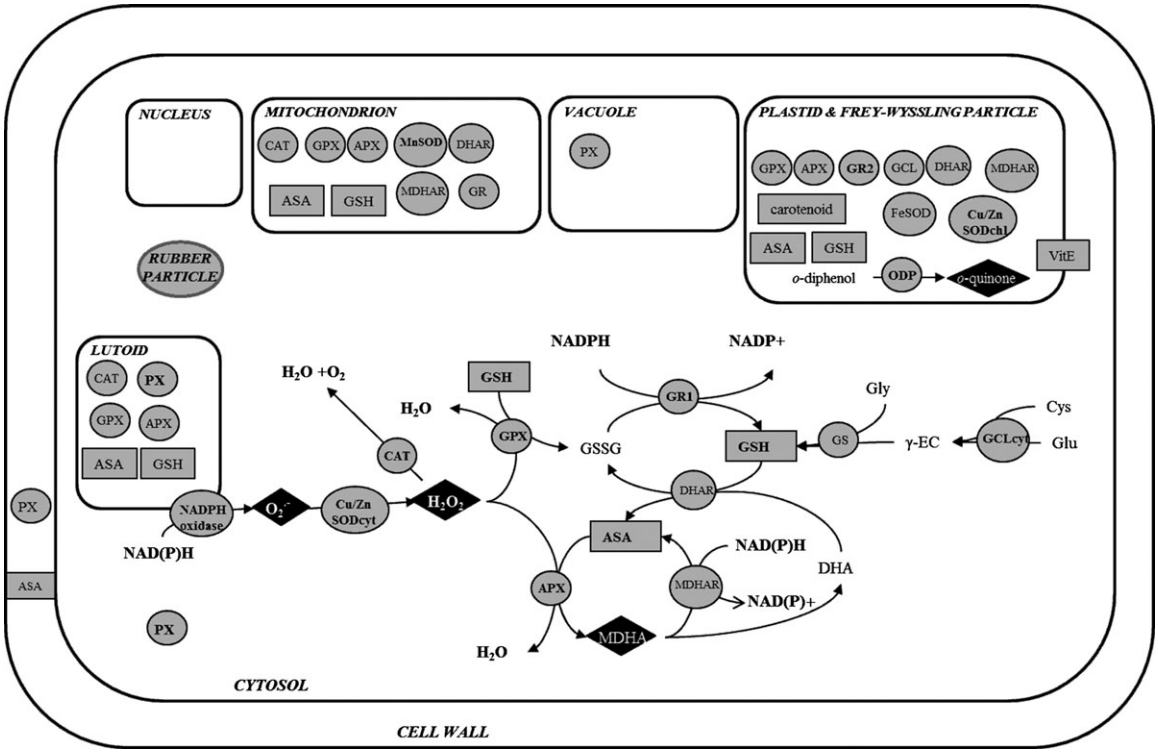

Figure 2. General scheme of ROS production and scavenging systems in latex cells. Enzymes are in grey circles, antioxidants in grey squares and ROS in black diamonds. Subcellular localization of enzymes and compounds is specified in normal letter according to Alscher et al. (2002), and in bold type when experimentally determined. CAT, catalase; PX, peroxidase; ASA, ascorbate; GSH, glutathione; APX, ascorbate peroxidase; GPX, glutathione peroxidase; MDHAR, monodehydroascorbate reductase; DHAR, dehydroascorbate reductase; GR, glutathione reductase; GCL, glutamate cysteine ligase; GS, glutathione synthetase; Gly, glycine; γ-EC, γ-glutamylcysteine; Cys, L-cysteine; Glu, L-glutamate; ODP, *o*-diphenol oxidase. The four vitamin E isoforms, namely α-tocopherol, α-tocotrienol, γ-tocotrienol and δ-tocotrienol, are specified as VitE, and they are assumed to be present in plastid membrane according to Munné-Bosch and Alegre (2002).

Interestingly, one gene encoding a GDP-D-mannose-3′,5′-epimerase was expressed at a higher level in a super-high-yielding tree (Tang et al. 2013). This super-high-yielding tree is more capable of lowering stress levels over time, thereby making it possible to invest more effort in the metabolic pathways related to latex regeneration. The antioxidant power of glutathione and ascorbate is also intensively regenerated by the enzymes of the glutathione–ascorbate cycle. Dehydroascorbate reductase (DHAR), GR (Jacob et al. 1984, Prevot et al. 1984*a*), cytosolic GR (Deng et al. 2014), ascorbate peroxidase (APX) and at least two glutathione peroxidases (GPXs) have been characterized (Clément et al. 2001, Dai et al. 2013). A gene encoding a GPX was upregulated during the first five tappings of re-opened rubber trees (Yujie 2011). An *APX* gene was upregulated in rubber clone CATAS8-79, in which latex regeneration was more effective than in clone PR107 (Chao et al. 2015*a*). The available NADPH content and the presence of certain inhibitors in situ, such as quinoid-type molecules, Cu^{2+} and Zn^{2+}, are likely to control GR activity physiologically (Jacob et al. 1984). GR activity was shown to be 10 times higher in latex than in lutoid (Prevot et al. 1984*a*). More recently, two *GR* genes were characterized (Deng et al. 2014, 2015). The *GR1* and *GR2* genes are expressed in latex and induced by ethylene, jasmonate, H_2O_2 and wounding treatment.

There are four vitamin E isomers in latex, namely α-tocopherol, α-tocotrienol, γ-tocotrienol and δ-tocotrienol (Dunphy et al. 1965, Whittle et al. 1966, Lee 1993, Yacob et al. 2012). The α-tocopherol is the saturated isoform of tocotrienols. γ-tocotrienol is the most abundant molecular variant in latex and all tocotrienols could amount to about 8% of total lipids (Dunphy et al. 1965, Chow and Draper 1970). Natural antioxidants in latex are probably involved in the quality of NR in fresh harvested latex, and during rubber maturation and processing. Oxidative degradation occurs during storage hardening of raw rubber (Morris 1991). Natural antioxidants might hamper such oxidation but are not sufficient in latex to protect the polymer. Vitamin E, phytosterols, phospholipids, phenols, betaines, proteins and some amino acids from the latex can act as antioxidants against oxidation in raw rubber (Altman 1948, Dunphy et al. 1965, Tirimanne et al. 1971, Musigamart et al. 2014). Among the latex antioxidants, vitamin E has been suggested as the main native antioxidant in raw rubber. The fat-solubility of vitamin E can help it to persist in raw rubber during processing (Liengprayoon et al. 2013) and it maintains antioxidant potency in vitro (Kamal-Eldin and Appelqvist 1996).

Table 1. ROS production and scavenging in the latex of *H. brasiliensis*.

Function	Subcellular localization	Evidence level	Reference
ROS production			
Polyphenol oxidase	Cytosol, B-serum	Enzyme activity	Tata and Edwin (1970)
	Unknown	Protein	Wang et al. (2015)
o-diphenol oxidase	Frey-Wyssling particles	Enzyme activity	Coupé et al. (1972)
NADPH oxidase	Lutoid membrane	Enzyme activity	Chrestin et al. (1984)
Peroxidase	Lutoids, cytosol	Enzyme activity	de Haan-Homans (1950); Tata and Edwin (1970); Coupé et al. (1972); Chrestin (1984); Wititsuwannakul et al. (1997)
	Unknown	Protein	Wang et al. (2015)
ROS-scavenging			
Catalase	Cytosol, B-serum	Enzyme activity	de Haan-Homans (1950); Tata and Edwin (1970); Coupé et al. (1972); Chrestin (1984)
Superoxide dismutase	Cytosol, B-serum	Enzyme activity	Chrestin (1984)
	Cytosol	Enzyme activity	Clément et al. (2001)
	Cytosol	Protein	Jiyan (2011)
	Unknown	Protein	Wang et al. (2015)
	Unknown	mRNA	Chao et al. (2015*a*)
	Cytosol	Transgenic plant	Leclercq et al. (2012)
Ascorbate peroxidase (APX)	Cytosol	Enzyme activity	Clément et al. (2001)
	Unknown	Protein	Wang et al. (2015)
	Unknown	mRNA	Putranto (2012)
	Cytosol	mRNA	Chao et al. (2015*a*, *b*)
Monodehydroascorbate reductase (MDHAR)	Unknown	Protein	Wang et al. (2015)
Dehydroascorbate reductase (DHAR)	Unknown	Enzyme activity	Clément et al. (2001)
	Unknown	Protein	Wang et al. (2015)
Glutathione peroxidase (GPX)	Cytosol	Enzyme activity	Chrestin (1984)
	Cytosol	Enzyme activity	Clément et al. (2001)
	Unknown	mRNA	Fan (2011)
Glutathione reductase (GR)	Cytosol	Enzyme activity	Jacob et al. (1984); Prevot et al. (1984*a*)
	Cytosol	mRNA	Deng et al. (2014)
Glutathione *S*-transferase	Unknown	Enzyme activity	Balabaskaran and Muniandy (1984)
	Unknown	Protein	Wang et al. (2015)
Ascorbate	Cytosol	1.1 mM	Archer et al. (1969)
Glutathione	Cytosol	0.3 mM	Archer et al. (1969)
Tocopherol/tocotrienol	Membrane	8% of lipids	Dunphy et al. (1965)
Ascorbate biosynthesis			
GDP-L-galactose phosphorylase (VTC2)	Unknown	mRNA	Fan (2011); Tang et al. (2013)
GDP-mannose-3anno epimerase	Unknown	mRNA	Tang et al. (2013)
Tocopherol/tocotrienol biosynthesis			
Geranylgeranyl reductase	Unknown	Protein	Wang et al. (2015)

Analysing the dynamic of tocotrienol was even suggested as the resistance parameter of rubber to oxidation during raw rubber processing (Musigamart et al. 2014).

Antioxidant defence enzymes, such as *SOD*, *CAT*, GPX and glutathione *S*-transferase (GST), are crucial for breaking down the harmful end-products of oxidative modification. Concomitant with an increase in respiration, tapped trees also enhanced the enzymatic ROS-scavenging system in soft bark tissues (Annamalainathan et al. 2001). Catalase and peroxidase activities were investigated in latex (de Haan-Homans 1950, Tata and Edwin 1970). About 60–80% of peroxidase activity was localized in lutoids and the rest in cytosol. About 50% of *CAT* activity was localized in some kind of particle (probably lutoids) and the rest in cytosol (Coupé et al. 1972). Peroxidases were also investigated in bark of rubber tree (Wititsuwannakul et al. 1997, Gopal and Thomas 2014). Considering the low affinity for H_2O_2 of *CAT*, which may only act to remove high H_2O_2 concentrations in case of oxidative burst, *APX* and GPX activities, with high affinity, are suitable for detoxification of low amounts of H_2O_2 (Clément et al. 2001). Recently, the down-regulation of a *HbAPX* gene by ethephon was suggested to disturb the redox homoeostasis in laticifer cells of rubber tree (Chao et al. 2015*b*).

Superoxide dismutase activity was first reported by d'Auzac et al. (d'Auzac et al. 1989). This enzyme is encoded by a multigene family consisting of a *MnSOD* (Miao and Gaynor 1993) and two Cu/Zn *SOD*s, a cytosolic isoform (Leclercq et al. 2012) and a chloroplastic form (Gébelin et al. 2013*a*). The *MnSOD*

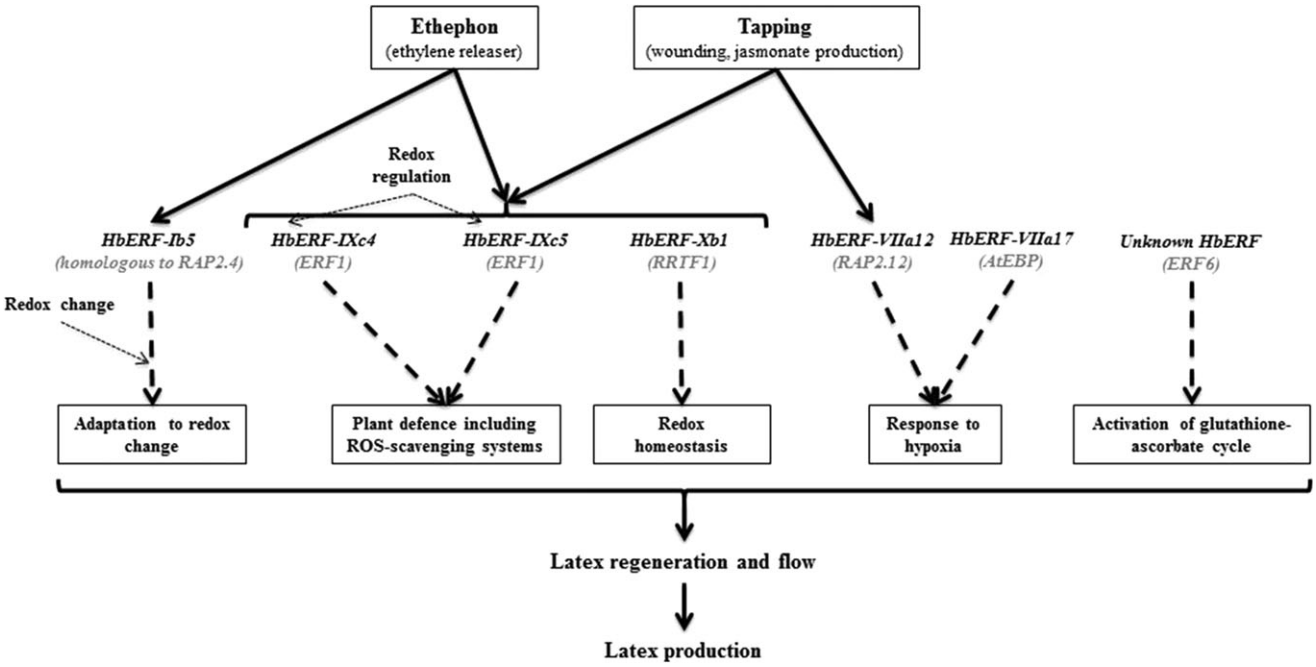

Figure 3. Working model of the regulatory network controlling redox systems and response to hypoxia in *Hevea* through ethylene response factors (ERFs). Black arrows: activation of function. Dashed arrows: assumption based on function demonstrated in Arabidopsis. Grey letters: ortholog gene in Arabidopsis based on phylogenetic analysis. Promoters of *HbERF-IXc4* and *HbERF-IXc5* genes harboured antioxidant responsive elements (AREs), suggesting redox regulation of their transcription.

gene was first upregulated and then downregulated in latex during the first five tappings of re-opened rubber trees (Jiyan 2011). Interestingly, a *SOD* gene was upregulated in rubber clone CATAS8-79, in which latex regeneration was more effective than in PR107 (Chao et al. 2015*a*). The *CAT* gene was first cloned by Kongsawadworakul et al. (Kongsawadworakul et al. 1997). Several other redox-related genes have been identified: thioredoxin H-type (Chow et al. 2007), hydrogen peroxide-induced metallothionein (HbMT2) (Zhu et al. 2010), thioredoxin and two amine oxidases (Yujie 2011). Lastly, a detoxifying enzyme, GST, was detected in a variety of tissues with a broad pH optimum between 8.5 and 9.5 (Balabaskaran and Muniandy 1984).

ROS-associated TPD affects latex production

Tapping Panel Dryness seriously affects the latex production of a rubber tree plantation. Tapping Panel Dryness refers to two syndromes (Putranto et al. 2015*b*). The first is related to overproduction of ROS and consequent cellular damage that can be reversible after resting trees without tapping (Das et al. 2002). The second form, called brown bast, involves histological changes and senescence mechanisms (de Faÿ and Jacob 1989*b*). Tapping Panel Dryness susceptibility depends on genetic and environmental factors. Overexploitation of rubber trees including a high tapping frequency and ethephon stimulation can cause early TPD occurrence associated with a decrease in thiol content (Putranto et al. 2015*b*).

The ROS generation and subsequent peroxidation of the cellular membrane system were first reported to be involved in latex flow stoppage by Cretin and Bangratz (1983). High NAD(P)H oxidase activity at the surface of lutoids was considered as the main source of ROS leading to peroxidative degradation of the unsaturated lipids of the lutoid membranes, then the release of factors involved in latex coagulation (Chrestin et al. 1984). Quinoid-type molecules and Cu^{2+} are activators of NADPH oxidase (Chrestin 1989). Quinoid-type molecules, such as plastoquinone and ubiquinol, are components of lutoids (Archer et al. 1969). The concentration of Cu^{2+} in lutoids is twice the concentration in cytosol (d'Auzac et al. 1982). The quinoid-type molecules and Cu^{2+} released from lutoids at the beginning of lutoid bursting probably inhibit GR activity but activate NADPH oxidase activity. In other words, ROS accumulation enhances the peroxidative degradation of lutoid membranes, which is a positive feedback to lutoid bursting. In a second step, ODP activity specifically expressed in Frey-Wyssling particles was noted in cytosol from TPD-affected trees revealing the lysis of Frey-Wyssling particles (Cretin and Bangratz 1983). Hevein was then shown to be involved in the agglutination of rubber particles (Gidrol et al. 1994). Another *Hevea* latex lectin-like protein present on the lutoid membrane, the small rubber particle protein, was reported to induce aggregation of rubber particles and lutoid membranes (Wititsuwannakul et al. 2008).

Typical TPD symptoms exhibit abnormally high NAD(P)H oxidase and peroxidase activities, but also a very low activity in ROS-scavenging enzymes such as *SOD* and *CAT* (Chrestin 1989).

This was confirmed on the bark of trees overstimulated with a high concentration of ethephon, which can generate higher concentrations of free radicals and exhibit lower *SOD* activity than in an untreated tree (Das et al. 1998). The *SOD* and GST protein contents decreased in latex after ethephon stimulation (Wang et al. 2015). Taken together with the protein accumulation of peroxidase and monodehydroascorbate peroxidase in ethephon-stimulated trees (Wang et al. 2015), this indicates that a high ethephon concentration is an ROS-related toxin for latex tissue. The expression of *CAT* and *MnSOD* genes can be stimulated by moderate ethylene treatment in a healthy tree but not in trees affected by TPD (Kongsawadworakul et al. 1997). By contrast *GR1* and *GR2* genes are upregulated in latex and bark of TPD-affected trees (Deng et al. 2014, 2015). Some other ROS-scavenging systems have been identified but not clearly characterized. For instance, inhibitors of NAD(P)H-quinone-reductase activity were suggested to be involved either directly in this enzyme inhibition or indirectly, by scavenging toxic oxygen produced by the reaction; the possibility of using these inhibitors in situ on the tapping panel was suggested (d'Auzac et al. 1986). Generally speaking, antioxidants and ROS-scavenging enzymes are related to the preservation of rubber production capacity (Lacote et al. 1998, Das et al. 2002).

Over the last decade a substantial effort has been made in understanding transcriptional regulation when TPD occurs. Expression of the *HbMyb1* transcription factor was significantly decreased in the barks of TPD trees (Chen et al. 2003). In another report, down-regulation of another Myb transcription factor and the thioredoxin H-type gene was shown in TPD trees (Venkatachalam et al. 2007). The suppression of stress-induced cell death by HbMyb1 was demonstrated in transgenic tobacco (Peng et al. 2011). Recent development of Next-Generation Sequencing technology has made it possible to identify both small RNAs and transcripts differentially expressed in trees affected by TPD (Gébelin et al. 2013*b*, Liu et al. 2015). According to the Gene Ontology annotations, 20 miRNA families are involved in regulating the expression of antioxidant activity genes (Gébelin et al. 2012). About 70 antioxidant activity genes were expressed in the bark of healthy and TPD-affected trees (Mantello et al. 2014, Liu et al. 2015). However, only seven antioxidant activity genes were predicted in latex (Wei et al. 2015).

Towards a comprehensive analysis of redox-related genes in *Hevea*

Characterization of the ethylene response factor (ERF) gene family in *Hevea* has led to the identification of several ERFs putatively involved in the regulation of redox genes (Piyatrakul et al. 2014). Their regulation by harvesting stress and their putative orthologs in *Arabidopsis* are presented in Figure 3. The *HbERF-Xb1* gene is orthologous to *RRTF1*, which has been described as the main node of the redox responsive co-expression network that controls a regulon responsive to a change in redox status (Khandelwal et al. 2008). Another ERF, RAP2.4a, was the first redox-modified transcription factor to be identified. This protein adopts conformational change according to the redox status. It binds to the target promoter of the *2CPA* gene as a dimer only under physiological redox conditions. Otherwise, under reducing conditions and oxidizing conditions, the inactive transcription factor stays as a monomer or an oligomer, respectively (Shaikhali et al. 2008). This gene should belong to *Hevea* ERF group Ia (Piyatrakul et al. 2014), but to date there are no identified orthologues in the *Hevea* transcriptome. The new complete genome version is expected to provide additional genes that could include this gene (Tang et al. 2016).

The biosynthesis of antioxidant compounds is also greatly controlled by ERF transcription factors. To date, no orthologous gene has been identified in rubber (Piyatrakul et al. 2014). In *Arabidopsis*, ERF98 activates the genes involved in the ascorbate biosynthesis pathway (Zhang et al. 2012). Some ROS-inducible ERFs have also been described in *Arabidopsis*. ERF6 is probably indirectly an activator of genes involved in the glutathione-ascorbate cycle, such as *DHAR1*, *APX4* and *CAT1*, because there is no GCC-box in the promoter of these target genes (Sewelam et al. 2013). Only promoters of two *ERF* genes, *HbERF-IXc4* and *HbERF-IXc5*, harbour an antioxidant responsive element *cis*-acting element revealing the putative response to the redox status of these genes (Piyatrakul et al. 2014, Putranto et al. 2015*a*). Although these two transcription factors are orthologues to ERF1, which controls a large panel of defence genes, there is no evidence for the activation of genes encoding ROS-scavenging enzymes (Piyatrakul et al. 2014). Interestingly, overexpression of these two *HbERF* genes conferred a better tolerance to abiotic stress (Lestari et al. Submitted).

Oxidative stress is induced by a wide range of environmental factors such as oxygen shortage. Generation of ROS in mitochondria was observed for hypoxia and especially for reoxygenation. In TPD-affected trees, the consumption of oxygen by NADH-cytochrome-*c*-oxidoreductase was particularly high and hypoxia condition was observed (Chrestin 1989). Genes *HbERF-VIIa12* and *HbERF-VIIa17* are putative orthologues to *RAP2.12* and *AtEBP*, which are involved in the activation of hypoxia-responsive genes through the N-end rule pathway (Piyatrakul et al. 2014). The AtEBP also confers resistance to hydrogen peroxide and heat treatments (Gibbs et al. 2011). Genes *HbERF-VIIa12* and *HbERF-VIIa17* are induced by tapping and constitutively highly expressed in latex, respectively, and might play a role in hypoxia response.

The genes involved in the ROS-scavenging system are also subjected to microRNA-mediated post-transcriptional regulations. Small RNAs have been deeply sequenced in *Hevea* in

various plant tissues and in the latex of healthy and TPD-affected trees (Gébelin et al. 2012, Lertpanyasampatha et al. 2012, Gébelin et al. 2013*b*). Several ROS-scavenging enzymes have been identified as targets of these microRNAs. The cleavage site by Hbmir398 has been experimentally validated for the chloroplastic CuZnSOD isoform only (Gébelin et al. 2012), and regulates the mRNA level of its target gene in response to salinity (Gébelin et al. 2013*a*). The *Rboh* transcripts have been predicted to be targeted by two miRNAs (HbmiR2914 and HbmiR476) (Gébelin et al. 2012).

Conclusions

This paper reviewed literature on the production and scavenging of ROS in latex cells and revealed that redox reactions are key functions for NR production and quality, as well as tolerance of biotic and abiotic stress. Several transcriptomic analyses showed transcriptional regulation of redox genes but we are far away from a comprehensive understanding of the regulation brought into play. The functional analysis of redox systems will necessitate an integration of proteomic and metabolomic information. This approach could lead to the identification of new factors, such as monoterpene, which might be a very effective molecule in protecting rubber plants against oxidative stress (JunWen et al. 2009). A role in the protection of raw rubber against thermo-oxidation has also been suggested for vitamin E. Given the large amount of vitamin E, and especially tocotrienol, these compounds could be exploited from waste serum generated during the processing of deproteinized NR (Sajari et al. 2014). Successful attempts have been made to engineer rubber plants with a high antioxidant capacity. Transgenic plants overexpressing *HbMnSOD*, cytosolic *HbCuZnSOD* and *EcGSH1* have been regenerated and characterized (Jayashree et al. 2003, Leclercq et al. 2012, Martin et al. 2015). Overexpression of the *HbCuZnSOD* and *EcGSH1* genes resulted in the production of fast-growing plants with greater tolerance of abiotic stress. Interestingly, these authors showed only that cytosolic *HbCuZnSOD* genes had no post-transcriptional regulation by microRNA398, which could affect the expression of these transgenes (Gébelin et al. 2012, Leclercq et al. 2012). As regards glutathione biosynthesis, the two *Hevea* genes encoding the glutamyl cysteine ligase are targeted by a microRNA but not the bacterial gene (*EcGSH1*) used in the experiment (Gébelin et al. 2013*a*). These transgenic plants accumulated three times more glutathione than wild-type plant material (Martin et al. 2015). Further applications of genetic engineering need to deal with the concerns of the public and NR supply chains regarding genetically modified organism (GMO) dissemination (Smith 2011). The public concern about GMOs should encourage researchers to use genetic variability in *Hevea* germplasm to improve tolerance of ROS-induced TPD and abiotic stress through conventional breeding programmes.

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